Mechanism of O_2-sensitive red cell properties

John S. Gibson

Department of Veterinary Medicine, Madingley Road, Cambridge, CB3 0ES.

Chu et al. [1] show here that oxygen, by changing the conformation of hemoglobin, modulates many red cell properties by altering its interaction with band 3, the major red cell membrane protein.

Several roles of oxygen are widely appreciated. Oxygen's participation in tissue metabolism via oxidative phosphorylation is well known, as is its carriage by red cell hemoglobin. Oxygen may also take part in the generation of reactive oxygen species whose threat is counteracted by various antioxidant systems, of which necessarily there are several inside red cells. These oxidants may also have important physiological functions, especially in cell signalling. A third essential role of oxygen, however, has so far eluded most standard accounts of respiration - namely its regulatory function in many other aspects of red cell physiology, central to its multiple roles in the circulation. In effect, oxygen constitutes a "molecular switch" to modulate red cell properties. This third role of oxygen is addressed by the paper of Chu et al. in the current edition of *Blood*. These authors present a major advance in the elucidation of the mechanism underlying this important role of oxygen.

The respiratory function of red cells involves three key proteins: hemoglobin, carbonic anhydrase and the anion exchanger (also known as band 3). Hemoglobin, of course, with its reversible oxygen binding, Bohr and Haldane effects is central to the transport of both blood gases. Of the several thousand million molecules of haemoglobin in each red cell, bulk cytoplasmic hemoglobin occupying around a third of red cell volume participates in this role. As we see in the present paper of Chu et al., the much smaller amounts of membrane-associated hemoglobin may, however, have a fundamentally different function.

Band 3, the anion exchanger, represents the most abundant protein in the red cell membrane, with a copy number of a few million. It exchanges Cl^- for HCO_3^-, and therefore participates in the carriage of blood gases. But, in addition, band 3 functions as a structural scaffold interacting with a variety of other proteins including glycolytic enzymes, the spectrin-actin cytoskeleton via ankyrin, and also haemoglobin [2-5]. The N-terminal tail of band 3 contains many acidic amino acid residues which are critical for these associations [2,6]. Change in oxygen tension over physiological values modifies these interactions, as well as other red cell functions, notably solute permeability [7,8].

The tetrameric shape of hemoglobin alters with oxygen tension, so that on deoxygenation a central cavity opens between the two alpha / beta dimers. 2,3-biphosphoglycerate binds at this site reducing hemoglobin's oxygen affinity. This cavity also interacts with the negatively charged N-terminus of band 3. As for 2,3-biphosphoglycerate, deoxygenated haemoglobin has a higher affinity for this N-terminus than when it is oxygenated. Thus deoxygenated hemoglobin has been shown *in vitro* to reduce the association of band 3 with some of its target proteins including glycolytic enzymes and ankyrin . This association therefore represents an obvious mechanism for how changes in oxygen tension may modulate a number of key red cell functions [7]. Deoxygenated hemoglobin has previously been shown to switch glucose metabolism from glycolysis to the pentose phosphate shunt [3,9] and also to reduce the interaction with ankyrin so weakening the cytoskeleton [5]. Until now, however, testing the veracity of this hypothesis *in vivo* has not been possible.

Low's group has long been interested in oxygen and red cell function. Their numerous important contributions to the field include work on the oxygen dependence of glucose metabolism and cytoskeletal integrity, in addition to much work on the structure of band 3 [2-6]. Here Chu et al. make use of transgenic mice expressing the N-terminus of human band 3 either containing, or

lacking, the binding site for deoxygenated hemoglobin to address its role *in vivo*. In a series of elegant experiments, they show that the presence of the band 3 binding site for human hemoglobin is required for mouse red cells to react to changes in oxygen tension with regard to three important red cell properties – glycolysis, integrity of the cytoskeleton and ATP release. They thus provide compelling evidence that reversible binding of hemoglobin to band 3, modulated through its oxygen dependent shape change, constitutes the molecular switch which controls these aspects of red cell behaviour.

As always, further questions arise. As Chu et al. comment, the important effects of oxygen on red cell solute permeability both in normal and diseased red cells - notably those form patients with sickle cell disease - awaits definition. In addition, it remains to be determined how animals lacking both band 3 and tetrameric hemoglobin, like lampreys [10], are nevertheless able to respond to oxygen at least with changes in solute permeability. Finally, the importance of this hemoglobin / band 3 interaction for the development and longevity of the red cell, and its various roles in the circulation, awaits elucidation. Notwithstanding, the current work represents a major advance in defining hemoglobin / band 3 interactions as an important molecular switch in regulation of red cell properties.

There are no conflicts of interest.

Figure legend

Figure 1. The molecular switch regulating oxygen-dependent red cell properties. 1a) Oxygenated, haemoglobin has a lower affinity for band 3, so that glycolytic enzymes and ankyrin are remain strongly bound and the ATP release channel is closed. 1b) On deoxygenation, haemoglobin has a higher affinity for band 3, displacing glycolytic enzymes and ankyrin and opening the ATP release channel. Glycolysis is stimulated, the cytoskeleton weakens, and ATP is released. The conformational change of haemoglobin on transition from oxygenated to deoxygenated states, via reversible band 3 binding, therefore represents the molecular switch regulating red cell properties.

Figure kindly provided by H. Chu & P. S. Low.

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